NOT TO BE FURTHER COPIED OR REPRODUCED

(* y VS x *)

11

Which Line?

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Suppose, at one extreme, that there is no perceptible tendency for the y's to be different for high x's than for low x's. Suppose more -- that there is also no perceptible tendency for the x's to be different for high y's than for low y's. When we fit a+bx to y, we will get a line of the form y = constant. When we fit c+dy to x, we will get a line of the form x = constant. These lines will be at right angles to each other, with either choice of axes. Clearly they cannot be the same line.

Let us return to the marriages-and-motor-vehicle-deaths example. Exhibit **1** summarizes the basic data and the residuals, for convenient reference.

Exhibit **2** goes forward with the detailed calculation, interchanging the roles of y and x.

We now have two polished fits

$$y \sim .811x + 48.$$

and

$$x \sim .978y - 40.8$$

The line $x = .978y - 40.8$ is the same as the line $y = 1.023x + 41.7$ (except for rounding), which is clearly not the same as $y = .811x + 48.8$. We need to look at these two lines in a number of ways.

In an optimistic vein, we begin by plotting the two lines against a background of the 49 points. Exhibit **3**, as it should, tells us little except that both lines are somewhat reasonable.

exhibit 2 of chapter 11

Fitting A Line to 1958 Marriages and Motor Vehicle Deaths With the Roles of x and y Interchanged

A) LOW-y PORTION -- y from -21 to 59 -- 16 points

		1st tilt	2nd tilt		level	
y	**x**	**x-y**	**-.03y**	**Diff**	**-39.8**	**Residuals**
40	23	-17	-1.2	-15.8	-39.8	24.0
-8	-63	-55	+.2	-55.2	-39.8	-15.4
-21	-9	12	+.6	11.4	-39.8	51.2
43	-2	-45	-1.3	-43.7	-39.8	-3.9
31	-15	-46	-0.9	-45.1	-39.8	-5.3
29	-22	-51	-0.9	-50.1	-39.8	-10.3
54	3	-51	-1.6	-49.4	-39.8	-9.6
28	-24	-52	-0.8	-51.2	-39.8	-11.4
0	-15	-15	-0.0	-15.0	-39.8	24.8
19	-37	-56	-0.6	-55.4	-39.8	-15.6
-13	-25	-12	+0.4	-12.4	-39.8	27.4
38	-25	-63	-1.1	-61.9	-39.8	-22.1
28	-17	-45	-0.8	-44.2	-39.8	-4.4
-11	-47	-36	+0.3	-36.3	-39.8	3.5
59	15	-44	-1.8	-42.2	-39.8	-2.4
14	-53	-67	-0.4	-66.6	-39.8	-26.8

					(cut)		(cut)
-2	1	-6	3	-6	37	-6	26	-2	6
-1	31	-5	3	-5	51126	-5	5015	-1	5015
-0	8	-4	7	-4	5654	-4	35942	-0	35942
0	0	-3	7	-3	6	-3	6	0	3
1	94	-2	2455	-2		-2		1	
2	988	-1	557	-1	752	-1	552	2	447
3	18	-0	92	-0		-0		3	
4	03	0	3	0		0		4	
5	49	1	5	1	2	1	1	5	1
		2	3	2					
					(full)		(full)
16		16		16		16		16	
4hH	-4	H	-31	H	-53h	H	-53.2	H	-13.4
8hM	28	M	-19h	M	-45h	M	-44.6	M	-4.8
4hH	39	H	-5h	H	-26h	H	-26.0	H	13.8
tri	22.8	tri	-18.8	tri	-42.8	tri	-42.1	tri	-2.3

Note: Portions were determined in panel C of exhibit 12 of chapter 10. Interlaced computations are in panel C of this exhibit.

(Cont'd)

exhibit 2 of chapter 11 (cont'd.)

C) CALCULATIONS ACROSS PORTIONS

		for portions; basis trimeans			(*)	apparent	trial
	At y =	22.8	76.2	107.8	(85.0)	(see *)	
	Adjusted:						
	raw x	-18.8		64.1	(82.9)	(0.97)	(1.00)
	1st adjusted	-42.8	-39.8	-45.1	(-2.3)	-0.027	(-0.03)
	2nd adjusted	-42.1	-37.2	-41.4	(0.7)	+.0058	polish
	same				[-120.7]	40.2	(-39.8)
	residuals	-2.3	2.6	-1.6	[-1.3]	-0.4	polish
	Fitted:						
1.00y	1st tilt	22.8	76.2	107.8			
-.03y	2nd tilt	-.7	-2.3	-3.2			
- 39.8	level	-39.8	-39.8	-39.8			
.97y - 39.8	total	-17.7	34.1	64.8			
	(residual)	(-2.3)	(2.6)	(-1.6)			
	restored x	-20.0	-36.7	63.2			
	Polished:						
(.970y - 39.8)	(total fit)	(-17.7)	(34.1)	(64.8)			
.008y	tilt polish	.2	.6	.9			
- 0.6	level for tilt	-.6	-.6	-.6			
- 0.4	level polish	-.4	-.4	-.4			
.978y - 40.8	polished fit	-18.5	33.7	64.7			
	polished res'l	-1.5	3.0	-1.5			

(*) In ( ), value for i-y portion minus value for lo-y portion -- followed by tilts, divisor 85.0.

In [ ], sum of values for 3 portions -- followed by levels, divisor 3.

(cont'd.)

11-6

exhibit 3 of chapter 11

The Data and the Two Lines

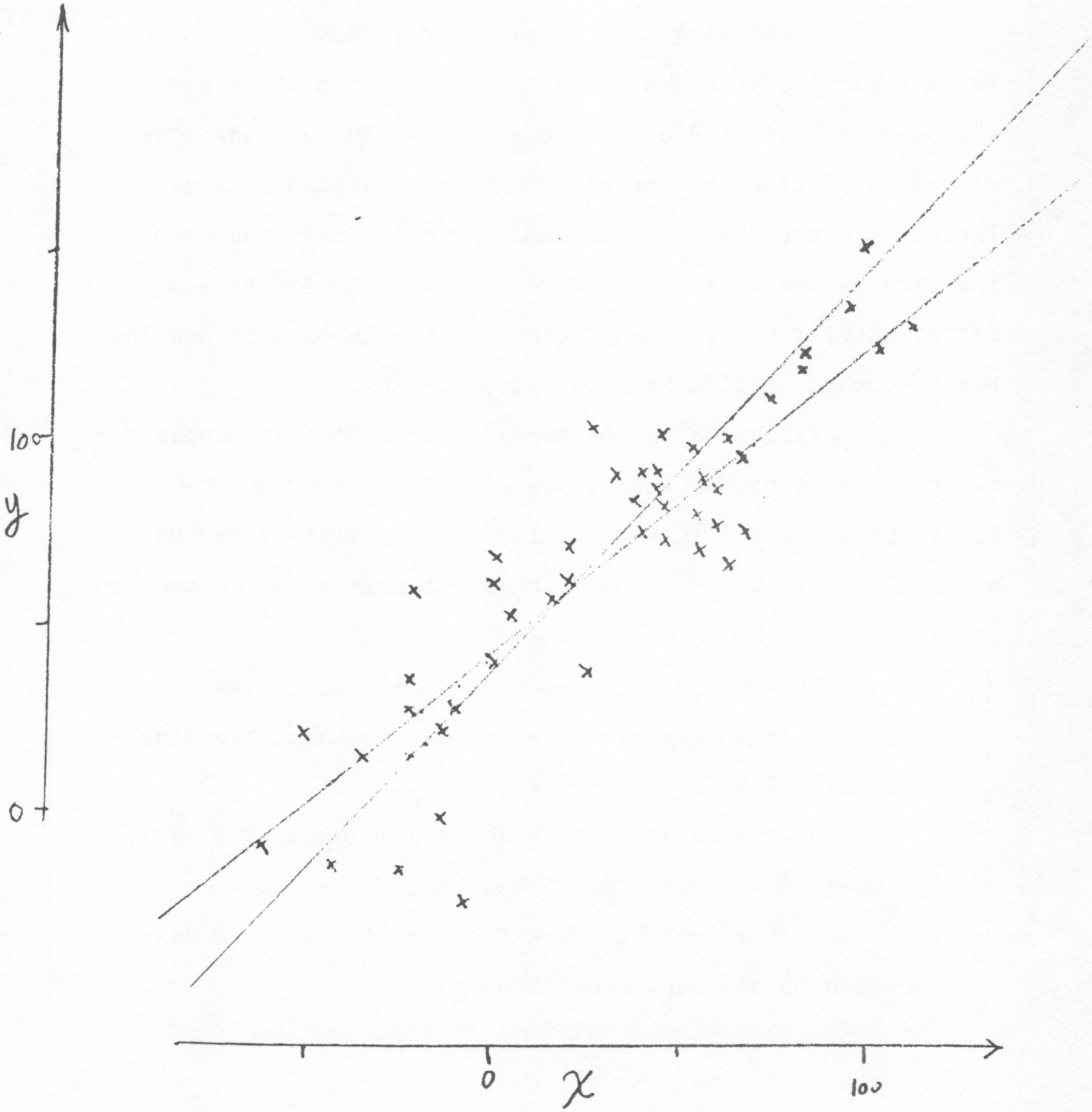


exhibit 4 of chapter 11

exhibit 13 of chapter 10 repeated

Residuals of y vs. Fit to y

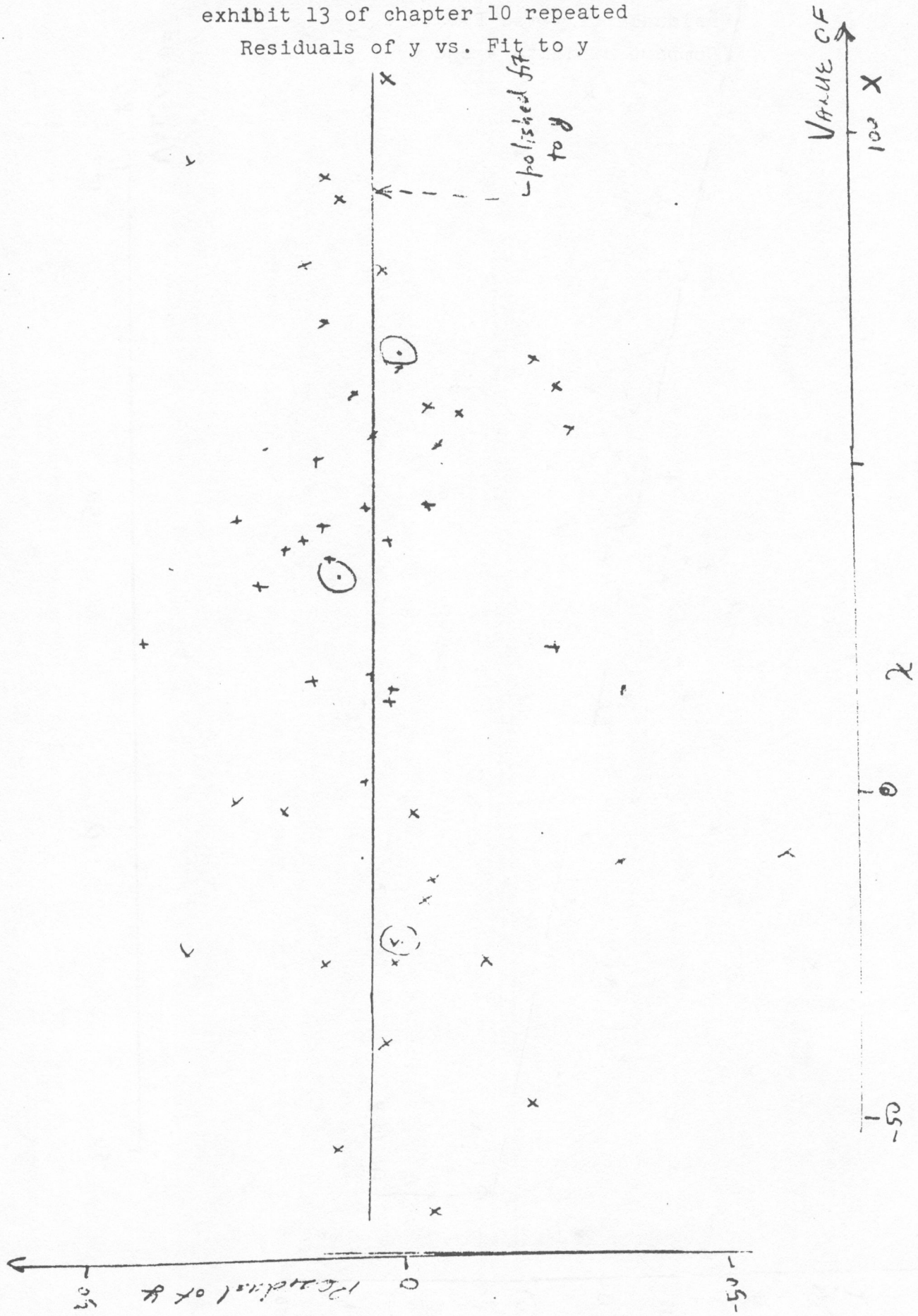
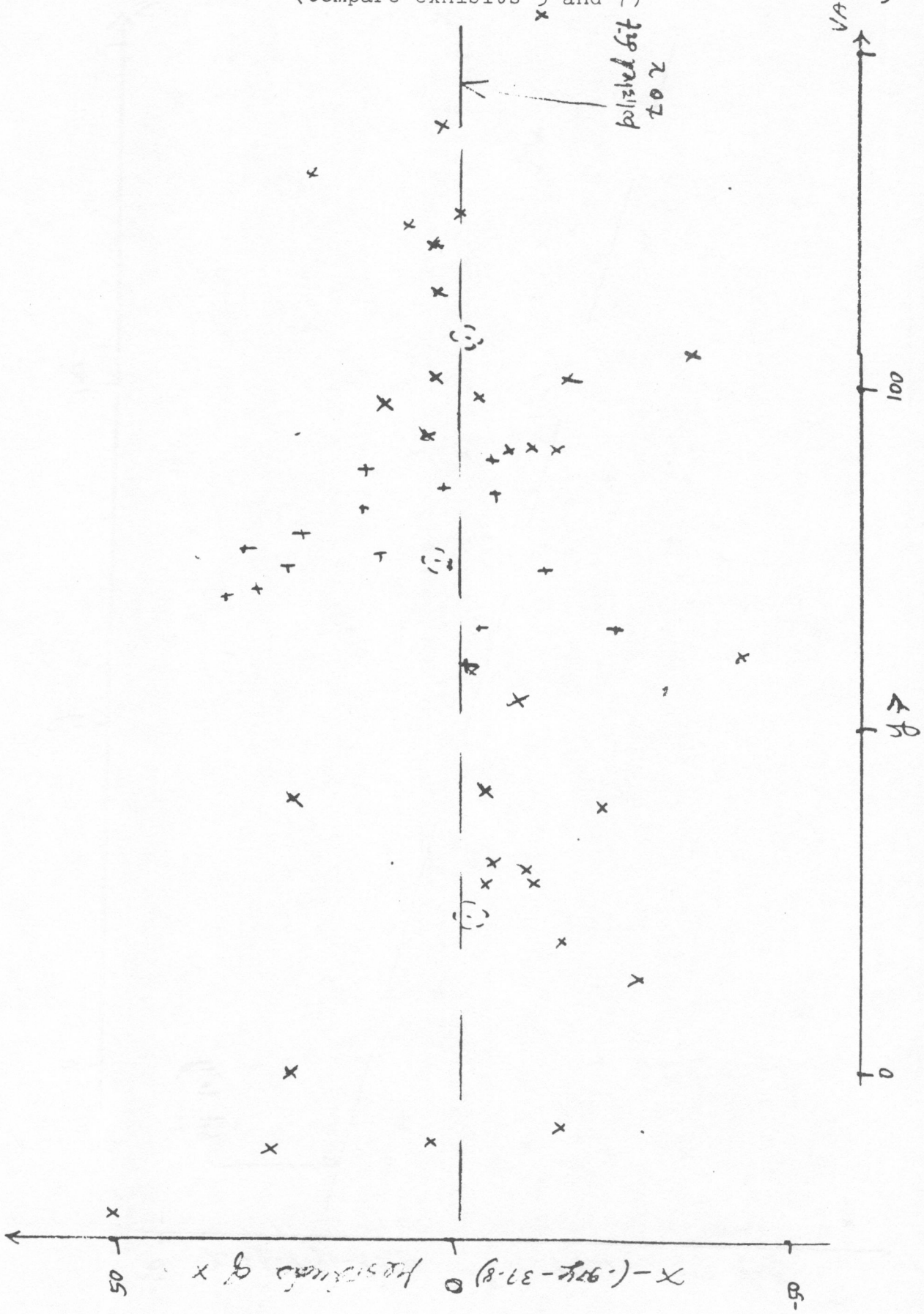


exhibit 6 of chapter 11

Residuals of x vs. Fit to x
(Compare exhibits 5 and 7)



Another way to look at the two competing lines is in terms of what happens for constricted ranges -- either of x or of y. If we look at exhibit 5, for example, and ask whether this fit is bearable for x's between -50 and 0, we have to admit that it is very unsatisfactory. Almost all the observations in this range of x are well above the fitted line. As exhibit 4 shows, the fit-to-y line is much more satisfactory for extreme x's than is the fit-to-x line.

If we interchange the roles of x and y, exhibits 6 and 7 show a similar phenomenon.

These facts let us state useful conclusions in a new form, namely:

- if we want an overall good fit, even if constricted ranges of x may be involved, we ought to use the fit to y.
- if we want an overall good fit, even if constricted ranges of y may be involved, we ought to use the fit to x.

Having come this far, we can now go further. Thinking that x is subject to selection is often associated with thinking that x is, in some sense, the cause of y. If x comes before y in time, for example, either people or impersonal causes could select out items with certain ranges of x, and then wait for the values of y to appear. Similarly the other way around.

- five New England states -- these include residuals of 31, 27, 24, 24 (four of 5 remaining positions) and 4 (Vermont).

It is probably sensible to add the four New England states. We can give plausible reasons for each of those on our list, though each explanation would require careful investigation before we believe it. D.C. has only low-speed-limit urban streets. Maryland probably was still a favorite for quick marriages. The New England states had low speed limits and strict enforcement. Speeds are known to be high -- and roads mountainous -- in Arizona and New Mexico. Florida is a place you go after you are married.

exhibit 8 of chapter 11 (cont'd.)

C) GEOGRAPHIC NEIGHBORS

State	x-residual	x	y	Relation
Delaware	-15	-63	-8	weak opposite
Pennsylvania	2	81	122	neutral
West Virginia	-2	15	59	neutral
Virginia	4	56	94	neutral
Arizona	-29	0	71	ALSO LOW *
Utah	-4	-17	28	neutral
Colorado	-1	17	60	neutral
Oklahoma	13	54	83	weak opposite
Texas	1	95	137	neutral
Georgia	11	66	98	weak opposite
Alabama	-11	39	93	weak similar

* One more state that may deserve special attention.

D) NEIGHBORS of ARIZONA

California	-13	98	155	weak similar
Nevada	-11	-24	28	weak similar

exhibit 9 of chapter 11

The 9-way Split of the States

	x from -63 to 0	x from 3 to 53	x from 54 to 110
y from -21 to 59	(-63,-8),(-9,-21),(-2,43) (-15,31),(-22,29),(-24,28) (-15,0),(-37,19),(-25,-13) (-25,38),(-17,28),(-47,-11) (-53,14)	(23,40),(3,54),(15,59)	
y from 60 to 90	(0,71),(-23,61),(-1,65)	(19,65),(17,60),(40,78) (19,74),(42,90),(37,85) (45,86),(45,76)	(62,70),(66,77),(56,74) (59,88),(54,83),(59,79)
y from 91 to 155		(39,93),(25,105),(33,93) (53,99),(43,103),(40,91)	(98,155),(66,98),(92,128) (62,102),(73,114),(110,133) (82,126),(81,122),(95,137), (56,94)

exhibit 11 of chapter 11

The Compromise Line Through the
Common-part Summary Point

A) LOW COMMON PART

for x		for y	
-6	3	-2	1
-5	3	-1	31
-4	7	-0	8
-3	7	0	0
-2	2455	1	94
-1	557	2	988
-0	29	3	18
		4	3
		5	
13		13	
4H	-37	H	-8
7M	-24	M	+19
4H	-15	H	+29
tri	-25	tri	+14.8

summary point: (-25 , 14.8)

B) HIGH COMMON PART

5	6	9	48
6	62	10	2
7	3	11	4
8	12	12	268
9	258	13	37
10		14	
11	0	15	5
10		10	
4H	66	H	102
7M	81.5	M	124
4H	95	H	133
tri	81	tri	120.2

summary point: (81, 120.2)

(Cont'd)

for marriages and motor vehicle deaths and closes with an interesting "round numbers" line for this specific example which is close to the compromise line and which is easy to remember.

* anchored lines *

Under some circumstances, it is natural to take a different approach to a compromise line, depending on a third variable to determine the portions that we use. In our example of the 49 states, the simplest and most natural third variable -- since both deaths and marriages involve people -- is the population of the state. Exhibit **12** arrays the states in order of 1960 census population and uses three portions defined on that basis to produce an

anchored line

whose equation is

$$y = .915x + 45.23$$

This line seems to be another satisfactory compromise.

11D. <u>A few words about measurement</u> (optional background)

Chapter 10 started with the assumption that the way to assess the tilt of y against x is to ask: "For what b is y-bx apparently untilted?". This flat statement appears more natural to some readers, and less natural to others. The present section provides some background about -- and, hopefully, some insight into -- what is going on in measurement in general.

exhibit **12** of chapter 11 (Cont'd)

C) SUMMARY POINTS -- for anchored thirds

high				mid				low			
	x		**y**		**x**		**y**		**x**		**y**
2	5	7	7	-0	1	4	0	-6	3	-2	1
3		8	8	0		5	9	-5	3	-1	31
4	30	9	9418	1	9597	6	550	-4	7	-0	
5	936	10	523	2	3	7	068944	-3	7	0	0
6	626	11	4	3	793	8	653	-2	35524	1	984
7	3	12	286	4	5250	9	330	-1	57925	2	898
8	12	13	37	5	946			-0	0	3	18
9	825	14		6	2			0	3	4	3
10		15	5							5	4
11	0									6	1
										7	1
16		16		17		17		16		16	
4hH	54h	H	96	**5**H	19	H	65	**4h**H	-31	H	4
8hM	16	M	104	**9**M	39	M	76	**8h**M	-22h	M	28
4hH	87	H	127	**5**H	45	H	85	**4h**H	-12	H	40h
tri	68.4	tri	107.8	tri	35h	tri	75h	tri	-22	tri	25.1

summary points: (-22.8,25.9), (35.5,75.5), (69.1,107.8)

D) ANCHORED LINE -- through end summary points

$$\text{tilt} = \frac{107.8-25.1}{68.4 - (-22} = \frac{82.7}{90.4} = .915$$

$$y \sim .915x + 45.23$$

how things are best compared. In the case of tilt, the things we are computing can be different enough in kind to make it worthwhile to recognize this difference in setting up our scheme of measurement.

The tilt of one straight line is easily compared with that of another straight line. The same is not true of two wiggly curves or two scattered trails of points -- to say nothing of two point clouds that are only somewhat elongated. Moreover, we know how to add tilts of lines. Since + and > together are enough, when well behaved (as they are here), to determine solid assignments of numbers, we can -- and should -- start with a qualitative notion of tilt for straight lines, and then extend this to more general patterns.

Our problem then -- given a wiggly curve, a scattered point trail, or a moderately elongated point cloud -- is to compare the tilt of this pattern with the tilt of various straight lines. We can do a low-to-medium grade job of this by drawing a straight line "through" the pattern before us. This process is often useful and ought never to be neglected. However, we can do better. Since better is available, our fundamental definition should take advantage of it.

The best way to compare the "tilt" of a wiggle, point-trail, or point-cloud with the tilt of a straight line is just to subtract the line from the pattern being studied.

urgently wanted an approximate answer. Using the number of marriages and one of the fitted lines should be better than guessing. Exhibit 13 shows the results of using various lines. The estimates vary from 698 to 753, a broad enough spread to make it reasonable for us to want to ask: which line is the proper one? But also a narrow enough spread to feel that the result may indeed be better than guessing.

The actual number was 719, in itself no real guide -- since we have but one instance -- to which line is proper.

How ought we to choose among the various lines? Circumstances can -- and do -- alter cases. But an "even-handed" attitude is likely to fit the true needs of many circumstances -- if not most. Moreover, it is easy to defend if an argument arises. By these words we mean a desire to be high about as frequently as low, whatever the range of x that may concern us. We know x -- so may others -- we can be neatly attacked if it has to be agreed that, because x is large -- or small -- we will tend to be high -- or low -- much more than half the time.

Since we are prepared to have x fixed -- or perhaps only to have it narrowed down -- we want to look at the y-residuals from any chosen line -- the residuals that show us the kind of errors we would make by using that line to go from x to y. To be "even-handed", we want a line that has about as many points above it as below it, not only overall, but also for chosen intervals of x.

We know which line this is. It is the line fitted to y, in this example the line we found first.

The instance is individual -- the principle is universal:

- to be even-handed, use the line that comes from fitting the unknown in terms of the known.

If the number of marriages had been missing instead, this same principle would apply, and we would use the line found by fitting a constant plus a constant times the number of motor vehicle deaths -- now the known -- to the number of marriages.

* an unsupported assertion *

Even-handedness can be conceived of in a number of quite different ways. We asked first for even-handedness in terms of x -- a little because we might be attacked in its terms, rather more because of a feeling of general justice and fairness. Had we wished to be even-handed in terms of y, we would have gone to the opposite extreme and used the line fitted to x.

Since the two lines are different, it follows that we cannot be even-handed with regard to both x and y at the same time.

A very interesting version arises when we want to be even-handed, not with regard to either the variable we know or the one we wish to assess, but with respect to some third variable. In dealing with marriages and motor vehicle deaths, for example, we might want to be even-handed with respect to general size of state, probably as made specific by its total population.

If this condition is met, and there is a measurable response to reasonable doses, then it is in principle possible to carry out an assay. In practice, the labor involved is likely to be too great for routine use unless someone has been ingenious enough to find a handy expression, y, of the response such that either:

- y depends linearly on x = log d, where d is dose of standard -- thus giving rise to a "parallel-line" assay

or

- y depends linearly on x = d, where d is dose of standard -- thus giving rise to a "slope-ratio" assay.

Given a standard, and a response that increases steadily with dose, then, once the condition is met, there is always an expression of the response that gives a parallel-line assay, and a different one that gives a slope-ratio assay. The question is not "Do there exist such expressions?". Rather it is "Have we found one?".

* only the small part *

We are engaged in exploratory data analysis. Accordingly we shall limit ourselves to the exploratory aspects of multiple-dose assays. These are only the beginning. In most actual assays, for instance, it is important to assess the precision of our results. Such questions are questions of confirmatory data analysis, however, and thus beyond our scope.

$$y \sim a + b(x+M) = (a+bM) + bx \ .$$

Thus bM is found as the y-difference between the two parallel lines.

Exhibit 14 shows the geometry of an ideal parallel-line assay. The vertical distance between the parallel lines is bM . The slope is b . The horizontal distance is thus M , as it should be, since M is, by definition, the difference in log doses for the same response.

* vitamin D examples *

Exhibit 15 treats an example. We find tilts for standard and unkonwn, accept the mean of these two tilts, adjust everything for this mean tilt, calculate the separation of the lines, which ought to tell us about

$$(\text{slope})\cdot(M)$$

divide by the accepted slope to learn about M, take antilogs to find the potency.

For simple data of this sort we are judging slopes by the differences in response of at the high dose and the low dose. It might pay us to look at these differences, as well as others. Exhibit 16 does this -- among other things -- using medians, trimeans and means to summarize the result. All the resistant analyses agree on

$$\text{hi-lo} = 5.0 \ .$$

So that we can expect fairly good adjusted values to be found by adding 2.5 at the low dose and subtracting 2.5 at the high dose.

exhibit 15 of chapter 11

Assay of Vitamin D Oil in Terms of Degree of Healing

A) DATA and SUMMARY

	Standard			Unknown		
Dose (in mg.)	2.5	5	10	2.5	5	10
Log dose = x	**0.4**	**0.7**	**1.0**	**0.4**	**0.7**	**1.0**
Litter **1**	2	8	8	3	9	7
" **2**	6	4	9	3	5	8
" **3**	4	6	12	4	6	9
" **4**	9	11	10	6	14	13
" **5**	10	15	17	8	8	10
" **6**	7	7	5	6	9	9
" **7**	4	10	13	5	11	13
" **8**	11	4	9	3	6	15
" **9**	2	9	14	5	8	6
" **10**	4	7	13	4	10	10
" **11**	12	10	9	15	18	15
" **12**	4	8	11	7	8	12
Sum	75	99	130	69	112	127
Mean	6.2	8.2	10.8	5.8	9.3	10.6

Entries are four times original grades of healing in the "line test".

B) From TILTS to POTENCY

Tilt $\frac{10.8-6.2}{1.0-0.4} = 7.7$ $\frac{10.6-5.8}{1.0-0.4} = 8.0$

mean tilt = 7.8

(7.8x)	3.1	5.5	7.8	3.1	5.5	7.8
Diff	3.1	2.7	3.0	2.7	3.8	2.8
mean		2.9			3.1	

Unknown - standard = 3.1 - 2.9 = 0.2 $\sim$ bM

$$M \sim \frac{0.2}{7.8} = 0.027$$

Since 0.027 is the log of 1.06, the unknown appears to be 1.06 times as potent as the standard.

C) SOURCE

K. H. Coward and E. W. Kassner, 1941. Biochem. J. 35 979-

Also used by C. I. Bliss at p. 498 of Statistical Methods in Vitamin Research, which is pages 448 to 610 of Vitamin Methods, Vol. 2, edited by P. Gyorgy, and published in 1951 by Academic Press,

Exhibit 17 shows adjusted values with medians over doses and comparisons within litter. The comparisons:

- are strikingly close to being symmetric.
- contain one extremely negative value and one extremely positive value. (The three-halves values, beyond which points, are said to be detached, are at -3.4 and +3.6; these extreme values are very much further out, at -6.5 and +7.5. They come from the two litters in which the response for one or the other middle dose is the largest for, respectively, standard or unknown.)
- have resistant central values quite close to 0.0.

It seems reasonable to conclude that:

- in this particular experiment, one positive stray matches one negative stray well enough to make the use of resistant techniques relatively close to the use of means.
- we have no reason to expect such balancing in other experiments under similar circumstances, so that resistant analyses should be very considerably more precise than analyses using means.

* importance of litters *

In this assay, as in so much well-conducted work with small animals, Coward and Kassner were careful about litters. They assigned one animal from each of 12 litters to each of the 6 combinations of material and dose -- hopefully in an

unbiased way. By doing this they became able to make comparisons within each litter and then summarize the result across litters. The great advantage is that each individual comparison is disturbed only by the lesser variations among the individuals of a single litter. (We all expect brothers and sisters to be more alike than unrelated individuals.) The larger differences between members of different litters are thus kept from perturbing the results of the assay. This is often very important.

* looking for curvature *

One thing we might be concerned about is a possibility of curvature. The grades of healing undoubtedly have least and greatest values. If we were to work with either too small or too large doses, we would expect the relation of y to x to be curved.

There are three simple ways to look for curvature. All look to see if the response appears to be a straight-line function of log dose. The first and second take advantage of the known spacing of log dose, asking how the mid-dose response lies with respect to the responses to the other two doses. Since our log doses are equally spaced, this means comparing the mid-dose response with the mean of the low-dose and high-dose responses, easily done by looking at the values of

exhibit 18 of chapter 11

Inquiry into Curvature
Second Approach

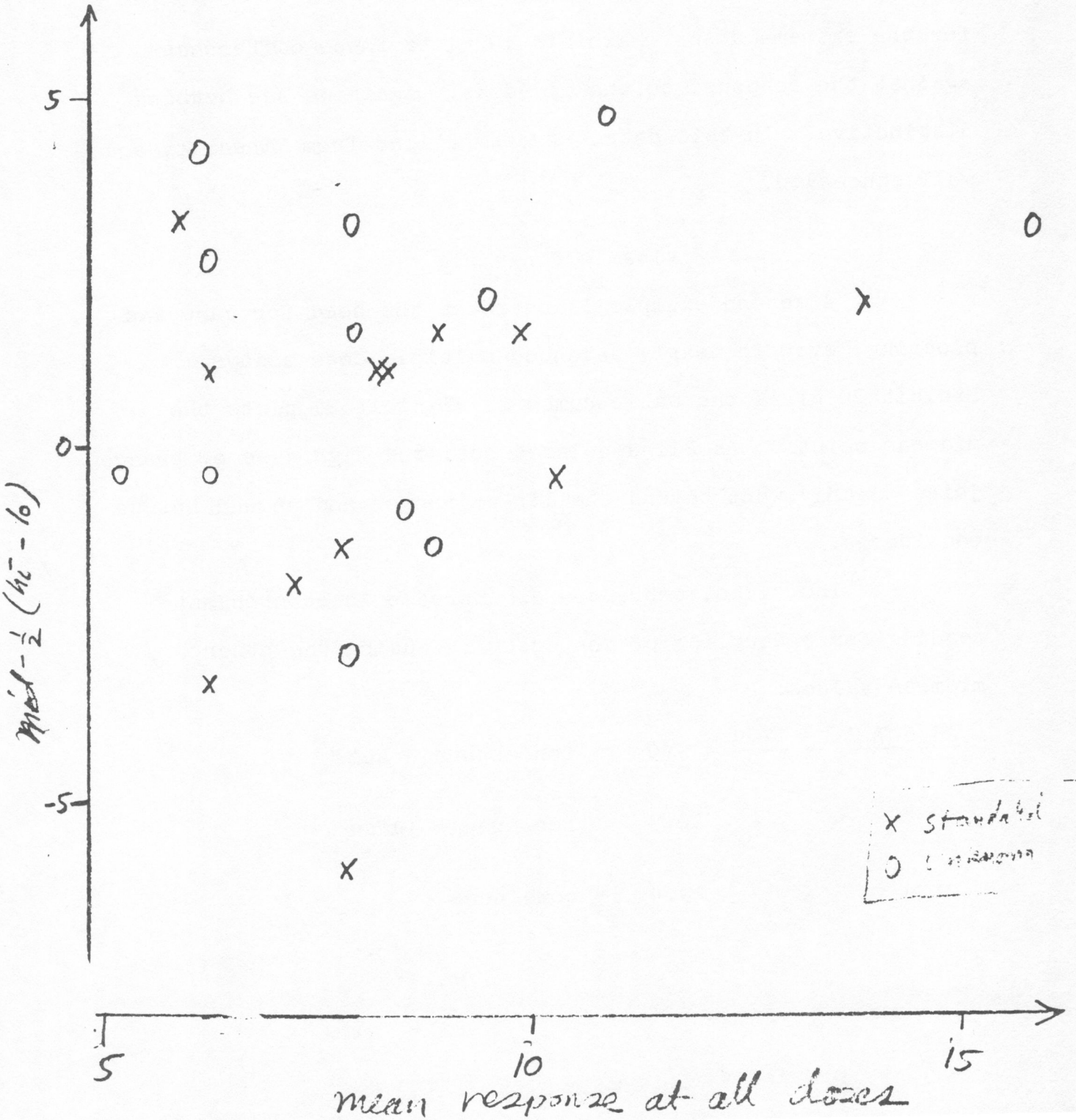


exhibit 19 of chapter 11
Inquiry into Curvature
Third Approach

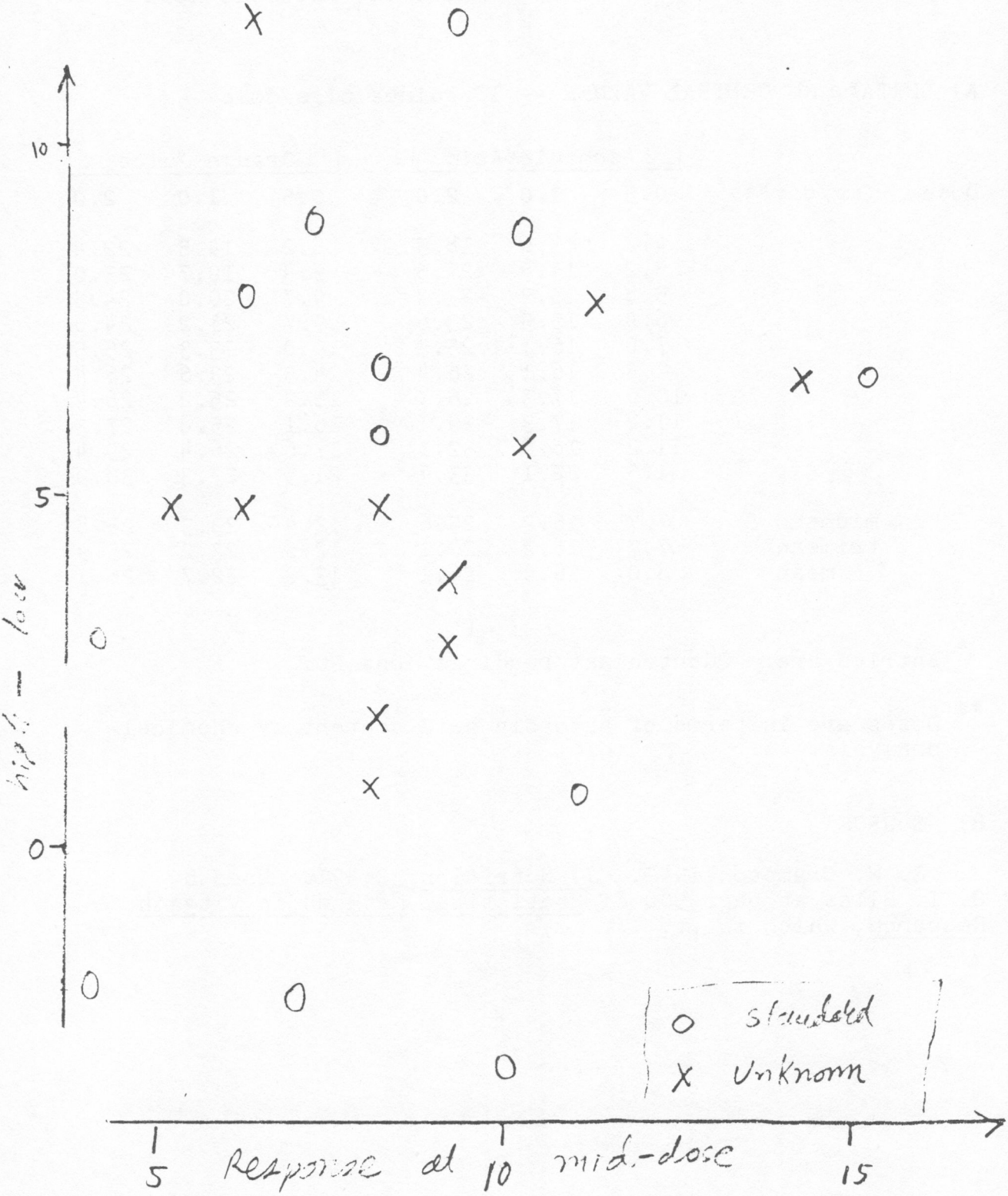
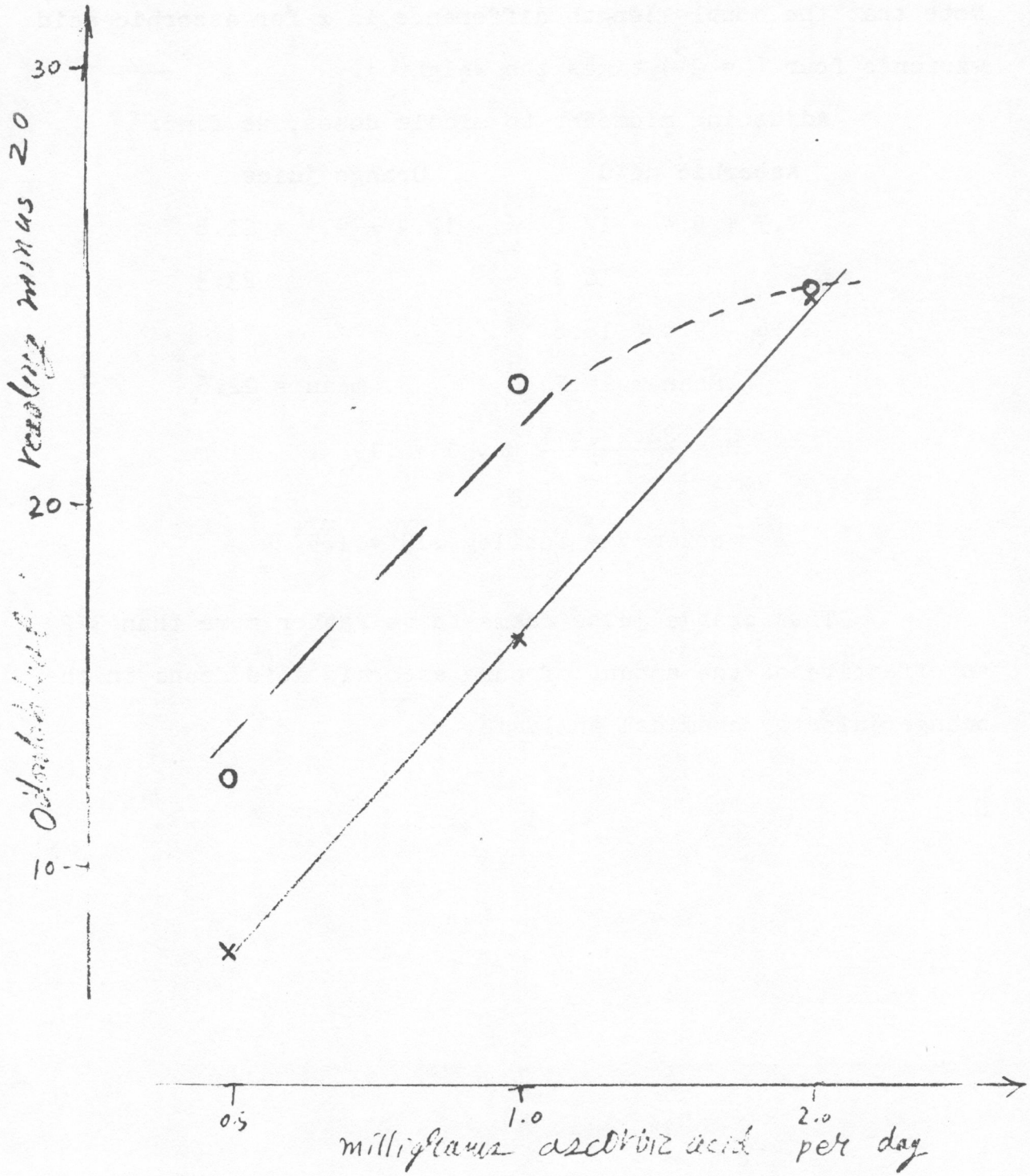


exhibit 21 of chapter 11

Midmean Points From exhibit 20



*****

11H. <u>How far have we come</u>?

We can fit

$$c+dy \quad \text{to} \quad x$$

just as we have fitted

$$a+bx \quad \text{to} \quad y \ .$$

In the conventional plot -- x horizontal, y vertical -- this new line is steeper than the old. It makes reasonable the residuals of x from a line in just the same way that the conventional line made reasonable the residuals of y from the line. When we know it is appropriate to make one set of residuals well-behaved, rather than the other, we know which line to use.

If we are to meet criticism of our even-handedness for specific ranges of x, we had better use the conventional line. (If for specific ranges of y, the steeper line will be needed instead.)

If we are to meet criticism on a general "large vs. small" basis, as measured by some third variable, we may do well to use a compromise line anchored by that variable.

Measurement is usually indirect -- we measure things by their effects, not in themselves. Thus a steady electric current, for example, is routinely measured either by the voltage it produces across a resistance -- or by how much a resistance heats up. Most measurement involves calibration.

*****

11K. <u>An extreme situation</u> (optional background)

One interesting situation that can occur is illustrated in exhibit 22, which was constructed by taking marriages and motor vehicle deaths for 21 states, 7 from each of the low-low, mid-mid, and high-high common parts of exhibit 9. A total of six lines are there fitted, one each with means, trimeans, and midmeans for y on x and for x on y. As panel J shows

- in this instance, which variable is fitted to which makes a negligible difference.
- in this instance, which central values we use makes a considerable difference, with trimeans closer to means than to midmeans.

The first occurrence reflects the rather unusual agreement of the x-portions and y-portions, which we caused artificially to provide an example.

The second occurrence reflects the skewed behavior of the low portion, which is perhaps most easily seen from the ordered values of y-x:

```
-12
 15H          mean = 36.0
 45         trimean = 39.8
 46M        midmean = 47.3
 51
 52H
 55
```

The midmean does, in this case, do a much better job of dealing with the bulk of the values. Even in batches as large as 7, we sometimes need the midmean rather than the trimean.

exhibit 22 of chapter 11 (Cont'd)

D) CALCULATION WITH MEANS

$$\text{tilt of y on x} = \frac{122.3-14.6}{83.3-(-21.4)} = \frac{107.7}{104.7}$$

$$= 1.03$$

$$\text{tilt of x on y} = \frac{83.3-(-21.4)}{122.3-14.6} = \frac{104.7}{107.7}$$

$$= .97$$

These give the same tilt, and will give the same line.

E) TRIMEAN TILT OF y ON x

	at x = -16.0	at x = 34	at x = 82	hi - lo	same 98	(trial)
y	20	77	122	102	1.04	(1.0)
y-x	40	44	39	-1	-.01	(take it)
(-.01x)	(.2)	(-.3)	(-.8)			
(y-.99x)	(39.8)	(44.3)	(39.8)	$\frac{\text{sum}}{3} = 41.3$		

$y \sim .99x + 41.3$

F) MIDMEAN TILT OF y ON x

	at x = -17	at x = 32	at x = 82	hi - lo	same 99	(trial)
y	19	79	123	104	1.05	(1.0)
y-x	47	45	39	-8	-.08	(-.08)
y-.92x	46.3	47.0	45.0	-1.3	-.012	(take it)
(-.01x)	(.2)	(-.3)	(-8)			
(y-.91x)	(46.1)	(47.3)	(45.8)	$\frac{\text{sum}}{3} = 46.4$		

$y \sim .91x + 46.4$

G) TRIMEAN TILT OF x ON y

	at y = 20	at y = 77	at y = 122	hi - lo	same 102	(trial)
x	-16	31	82	98	.96	(1.0)
x-y	-40	-44	-39	1	.01	(take it)
(.01y)	(.2)	(.8)	(1.2)			
(x-1.01y)	(-40.2)	(-44.8)	(-40.2)	$\frac{\text{sum}}{3} = -41.7$		

$x \sim 1.01y - 41.7$

(Cont'd)

Note also that it required two unusual values out of seven, both to distort the trimean and to keep even the unusual points from being outside -- the condensed notation being

dh	37
S	-22
S	89
out	no
out	no

The reader is not supposed to draw a heavy moral from this example. If he thinks about it long enough, he may draw some light considerations.

exhibit 23 of chapter 11

Titer Per Tube in Milliliters of 0.1N NaOH

(Riboflavin assay, entries are totals of 2 tubes)

Dose (µg/tube)	Standard	Unknown	Coded dose
.283	9.85	8.60	-7
.400	12.60	11.10	-5
.566	15.95	14.45	-3
.800	17.00	16.70	-1
1.130	20.70	19.05	1
1.600	23.00	20.75	3
2.260	27.30	25.05	5
3.200	27.25	27.55	7

exhibit 25 of chapter 11

Healing Score in Assay of Vitamin D Oils
(see text)

	Standard		Unknown A		Unknown B	
Litter	**4**	**8**	**4**	**8**	**4**	**8**
1	8	10	2	10	2	10
2	4	8	4	8	4	8
3	0	10	6	8	3	10
4	4	10	8	10	6	10
5	4	10	6	10	3	8
6	0	8	4	8	0	10
7	4	6	2	8	4	10
8	4	4	0	6	1	4
9	1	10	2	8	4	10
10	8	12	3	12	2	12
11	3	6	2	4	2	4
12	3	10	2	10	6	12

Bold figures are dosage levels.

*****

11R. References

* References related to fundamental measurement *

N. R. Campbell 1928. An account of the principles of Measurement and Calculation. 293 pp. London: Longmans, Green. (This book is harder to find than Campbell's Physics, the Elements, Vol. 1, which was reprinted by Dover as Foundations of Science: The Philosophy of Theory and Experiment, New York, 1957.)

For a more recent symposium on measurement, see: Measurement: Definitions and Theories. 274 pp. New York: John Wiley & Sons, Inc., edited by C. West Churchman and Philburn Ratoosh.

Recent attention to what is called simultaneous conjoint measurement began with a rather technical paper, whose opening sections may possibly prove readable:

R. Duncan Luce and John W. Tukey 1964. Simultaneous Conjoint Measurement: A New Type of Fundamental Measurement, J. Math. Psychol., 1-27.

11

Which Line?

This is an advanced chapter. We hesitate to mark it optional, for its concepts are important. Yet pressure of time may well, unfortunately, cause its skipping.

Fitting a straight line may seem easy in principle, though not so easy in practice -- especially when compared with stem-and-leaf based techniques for working with batches. One lesson this chapter teaches is that this is far from being so. There are many important choices in fitting a line that we have not met -- broad choices of principle, not just narrow choices of practicality.

11A. The other, steeper line

We have followed custom, and fitted

$$a+bx \quad \text{to} \quad y$$

It is, of course, always quite possible to interchange x and y. Once this is done, we will fit

$$c+dy \quad \text{to} \quad x$$

Until we stop and think, we might expect to find the same line from both approaches. Either thought, or an example, or both, however, teaches us that we cannot hope for this.

exhibit 1 of chapter 11

The 49 State Example Re-summarized

(mostly from exhibit 12 of chapter 10)

state	x	y	residual of y	residual of x*
Ala	39	93	16.4	-11.4
Ariz	0	71	24.8	-29.1
Ark	19	65	4.0	-4.2
Calif	98	155	30.4	-12.6
Colo	17	60	0.5	-1.4
Conn	23	40	-24.1	24.0
Del	-63	-8	-3.8	-15.4
D. C.	-9	-21	-60.0	51.2
Fla	25	105	39.7	-35.0
Ga	66	98	-1.0	10.7
Ida	-2	43	-1.6	-3.9
Ill	92	128	8.2	7.6
Ind	62	102	6.2	2.7
Iowa	40	78	0.6	4.1
Kans	19	74	13.4	-13.2
Kent	42	90	11.0	-5.5
La	33	93	21.0	-17.4
Maine	-15	31	-3.2	-5.3
Md	62	70	-25.8	33.9
Mass	66	77	-22.0	31.0
Mich	73	114	10.4	2.2
Minn	37	85	9.9	-5.6
Miss	56	74	-17.0	24.0
Mo	53	99	11.5	-3.2
Mont	-22	29	0.4	-10.3
Nebr	3	54	5.5	-9.6
Nev	-24	28	1.0	-11.4
N. H.	-15	0	-34.2	24.8
N. J.	59	88	-5.4	13.4
N. M.	-23	61	32.9	
N. Y.	110	133	-1.2	20.8
N. Car	43	103	23.3	-17.1
N. Dak	-37	19	2.4	-15.6
Ohio	82	126	14.2	-0.4
Okla	54	83	-6.4	13.3
Oreg	-1	65	17.8	-24.2
Penna	81	122	1.0	2.5
RI	-25	-13	-13.2	27.4
S. Car	59	79	-14.4	22.0
S. Dak	-25	38	11.8	-21.9
Tenn	45	86	4.7	1.4
Tex	95	137	14.8	1.9
Utah	-17	28	-4.6	-4.4
Vt	-47	-11	-19.6	3.5
Va	56	94	3.0	4.6
Wash	45	76	-5.3	11.1
W. Va	15	59	1.1	-2.4
Wisc	40	91	13.6	-8.5
Wyo	-53	14	10.2	-26.8

* Brought forward from exhibit12 for more complete reference.

exhibit 2 of chapter 11 (Cont'd)

B) HIGH-y PORTION -- from 91 to 155 -- 16 points

		1st tilt	2nd tilt		level	
y	**x**	**x-y**	**-.03y**	**Diff**	**-39.8**	**Residuals**
93	39	-54	-2.8	-51.2	-39.8	-11.4
155	98	-57	-4.6	-52.4	-39.8	-12.6
105	25	-80	-3.2	-76.8	-39.8	-37.0
98	66	-32	-2.9	-29.1	-39.8	10.7
128	92	-36	-3.8	-32.2	-39.8	7.6
102	62	-40	-3.1	-36.9	-39.8	2.9
93	33	-60	-2.8	-57.2	-39.8	-17.4
114	73	-41	-3.4	-37.6	-39.8	2.2
99	53	-46	-3.0	-43.0	-39.8	-3.2
133	110	-23	-4.0	-19.0	-39.8	20.8
103	43	-60	-3.1	-56.9	-39.8	-17.1
126	82	-44	-3.8	-40.2	-39.8	-0.4
122	81	-41	-3.7	-37.3	-39.8	2.5
137	95	-42	-4.1	-37.9	-39.8	1.9
94	56	-38	-2.8	-35.2	-39.8	4.6
91	40	-51	-2.7	-48.3	-39.8	-8.5

					(cut)		(cut)
9	383941	2	5	-8	0	-7 4	-3 7
10	523	3	93	-7		-6	-2
11	4	4	30	-6	00	-5 1276	-1 1277
12	862	5	36	-5	471	-4 308	-0 308
13	37	6	62	-4	016412	-3 267775	0 722214
14		7	3	-3	268	-2 9	1 0
15	5	8	21	-2	3	-1 9	2 0
		9	825				
		10					
		11	0				

					(full)		(full)
16		16		16		16	
4hH	96	H	41.5	H	-55.5	H -51.8	4hH -12.0
8hM	104	M	64	M	-43	M -39.0	8hM 0.8
4hH	127	H	87	H	-39	H -36.0	4hH 3.8
tri	107.8	tri	64.1	tri	-45.1	tri -41.4	tri -1.6

(cont'd.)

exhibit 2 of chapter 11 (Cont'd)

D) MID-y PORTION -- y from 60 to 90 -- 17 points

		1st tilt	2nd tilt		level	
y	**x**	**x-y**	**-.03y**	**Diff**	**-39.8**	**Residuals**
71	0	-71	-2.1	-68.9	-39.8	-29.1
65	19	-46	-2.0	-44.0	-39.8	-4.2
60	17	-43	-1.8	-41.2	-39.8	-1.4
78	40	-38	-2.3	-35.7	-39.8	4.1
74	19	-55	-2.2	-52.8	-39.8	-13.2
90	42	-48	-2.7	-45.3	-39.8	-5.5
70	62	-8	-2.1	-5.9	-39.8	33.9
77	66	-11	-2.3	-8.7	-39.8	31.1
85	37	-48	-2.6	-45.4	-39.8	-5.6
74	56	-18	-2.2	-15.8	-39.8	24.0
88	59	-29	-2.6	-26.4	-39.8	13.4
61	-23	-84	-1.8	-82.2	-39.8	-42.4
83	54	-29	-2.5	-26.5	-39.8	13.3
65	-1	-66	-2.0	-64.0	-39.8	-24.2
79	59	-20	-2.4	-17.6	-39.8	+22.2
86	45	-41	-2.6	-38.4	-39.8	1.4
76	45	-31	-2.3	-28.7	-39.8	11.1

				(cut)			(cut)
5		-8	4	-8	2	-4	2
6	5015	-7	1	-7		-3	
7	18404796	-6	6	-6	84	-2	94
8	5836	-5	5	-5	2	-1	3
9	0	-4	63881	-4	4155	-0	4155
		-3	81	-3	58	0	41
		-2	990	-2	668	1	331
		-1	18	-1	57	2	42
		-0	8	-0	58	3	31
				(full)			(full)
17		17		17		17	
5H	70	H	-48	H	-45.4	M	-5.6
9M	76	M	-41	M	-38.4	H	1.4
5H	83	H	-29	H	-26.4	M	13.4
tri	76.2	tri	-39.8	tri	-37.2	tri	2.6

(It ought to, since looking through the back of the page at the light interchanges x and y.)

To learn more we must be more specific. This means:

- showing only one line at a time.
- choosing what we plot carefully.

We have already seen, in exhibit 13 of chapter 10 -- now repeated as exhibit 4 of this chapter -- how well the line fitted to y looks in comparison with the residuals of y. Exhibit 5 shows the same residuals of y and the other line -- the line fitted to x. This line is obviously far from a satisfactory fit, with many points in the upper left and lower right corners pulling hard to twist it back.

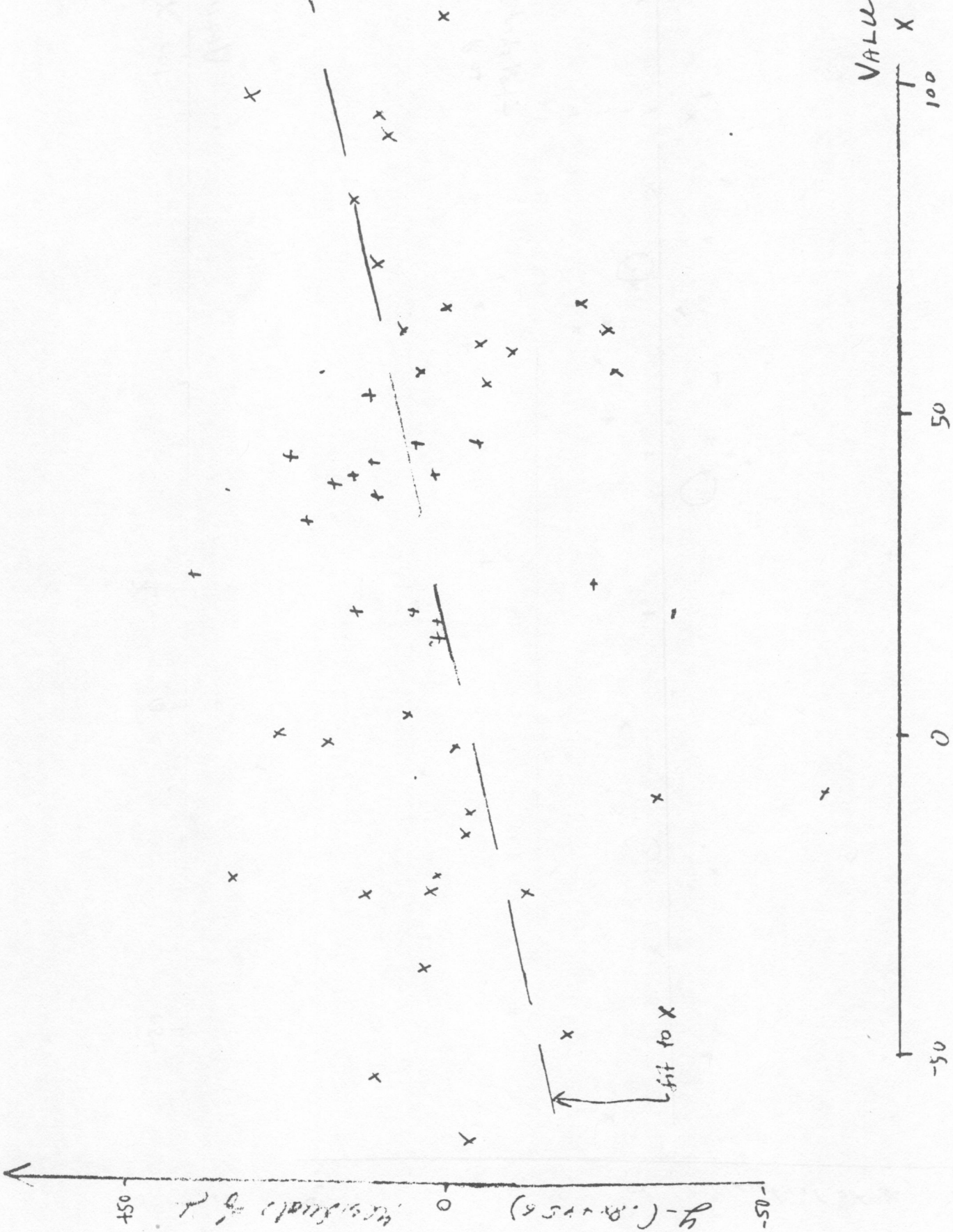
Exhibit 6 is the parallel to exhibit 4, being for residuals of x rather than residuals of y. Now the line fitted to x appears quite satisfactory. Exhibit 7 is the parallel to exhibit 5, showing the residuals of x but the line fitted to y. This line is now unsatisfactory.

In summary then, we stand about as follows:

- in an x-y plot, we can live with either line -- and might like to compromise.
- if we are thinking about residuals of y only, we need to use the line fitted to y.
- if we are thinking about residuals of x only, we need to use the line fitted to x.

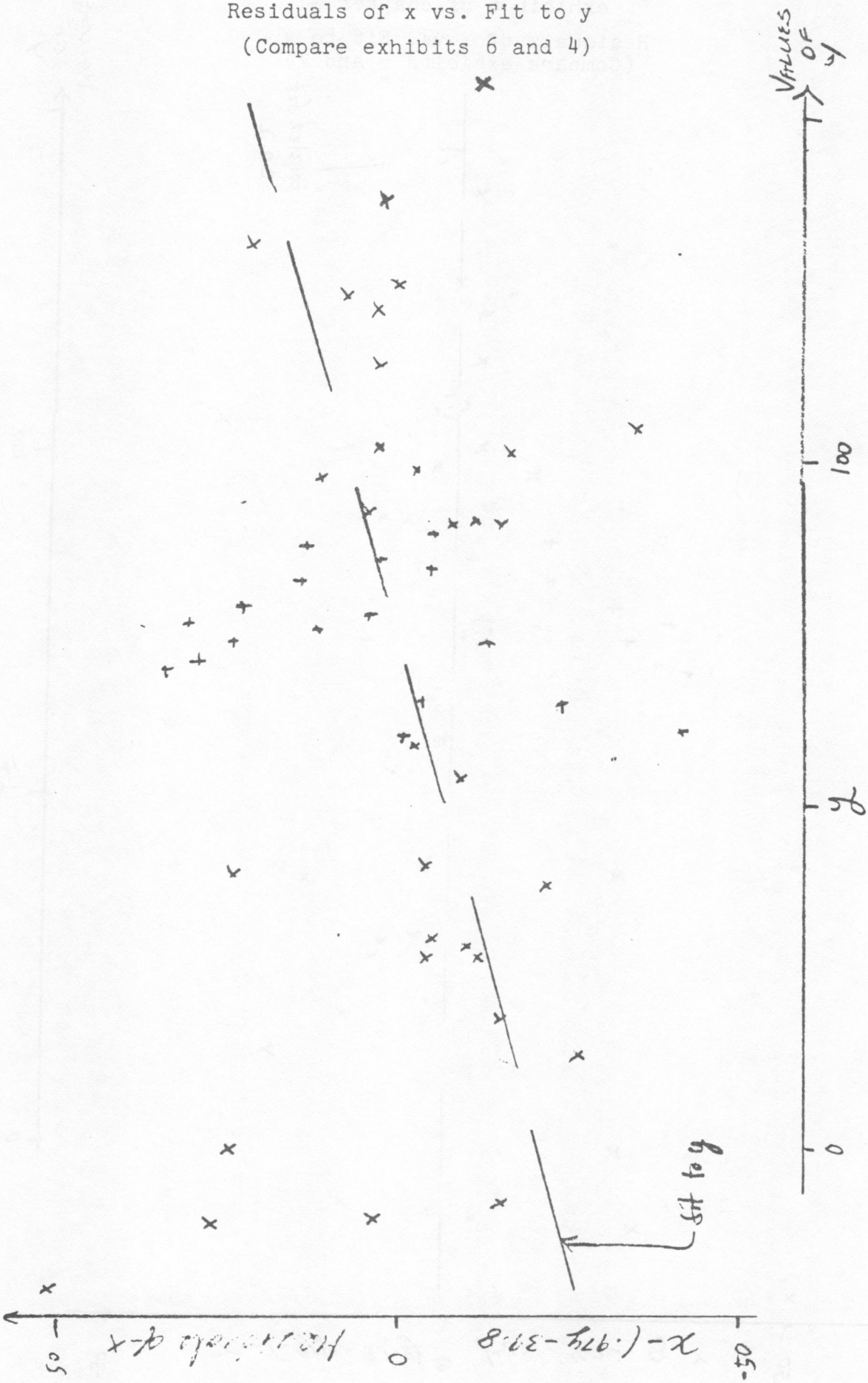
exhibit 5 of chapter 11

Residuals of y vs. Fit to x
(Compare exhibits 4 and 6)



Residuals of x vs. Fit to y

(Compare exhibits 6 and 4)



Accordingly, it is reasonable to say:

- if we think of x as the cause of y, we are likely to want to use the line fitted to y.

- if we think of y as the cause of x, we are likely to want to use the line fitted to x.

The first of these applies, in particular, to the indirect measurement -- or assay -- of a value of x by measuring a value of y. (We will say more about this when we come to sections 11E and 11F.)

11B. Residuals from the x-line

In this example, it is just as natural to look at the residuals of x -- calculated from the line fitted to x -- as it was to look at the residuals of y -- calculated from the line fitted to y. We can look at them in the same detail. Exhibit 8 shows the results of a parallel analysis.

This time fewer states fall outside:

- New Mexico and Arizona low (corresponding to high for a y-residual.
- Florida low.
- D.C. and Maryland high.

Those that did not reappear include:

- California, Oregon, and Texas not quite low enough.
- Alabama the same.

exhibit 8 of chapter 11

Analysis of all 49 residuals of exhibit 2
(compare exhibit 14 of chapter 10)

A) STEM-and-LEAF DISPLAY -- individual residuals cut to integers, as collected from panels A, B and D of exhibit 2

1	-4	2	**2**	
2	-3	7	**7**	
6	-3	9642		9642
to 15	-1	775532110		775532110
✓ 49 12	-0	985554433210		985554433210
to 22	0	112223447		112223447
13	1	0133		0133
3	2	026667		026667
3	3	13	**3**	1
	4			
1	5	1	**1**	

outside

49		dh	21
13H	-11	S	-32(2)
13H	10	S	31(2)

B) The OUTSIDE RESIDUALS

State	**x-residual**	**x**	**y**
New Mexico	-42	-23	61
Florida	-37	25	105
Maryland	33	62	70
District of Columbia	51	-9	-21

(cont'd.)

11C. <u>How can there be two lines?</u>

We have seen that there are two lines. That we can find them separately. That they each serve their own purpose. We have not yet tried to make clear how two lines come to be, or whether there are further alternatives.

Exhibit **9** shows the simultaneous two-way division of the points for the 49 "states" into x-portions and into y-portions. A total of 13 points are simultaneously in low-x and low-y. When we go to the whole of either the low-x or low-y portions, these 13 are joined by three others, a different three in each case. Each three pulls the summary point a little its way.

Only 10 points are in both high-x and high-y portions. In either whole portion they are joined by six others. It is reasonable that corresponding summary points are still further separated.

Exhibit **10** shows the data points and the summary points -- trimeans of coordinates -- for:

- each separate set of portions.
- the two common parts -- low-x-and-low-y and high-x-and-high-y.

We see our anticipations generally fulfilled, with the summary points for the individual portions so placed with regard to the common-part summary points as to twist one line one way and one the other.

When a compromise line is wanted -- which is not often -- we do fairly well by using the line through the common-part summary points. Exhibit **11** shows the calculation

exhibit 10 of chapter 11

Individual and Summary Points

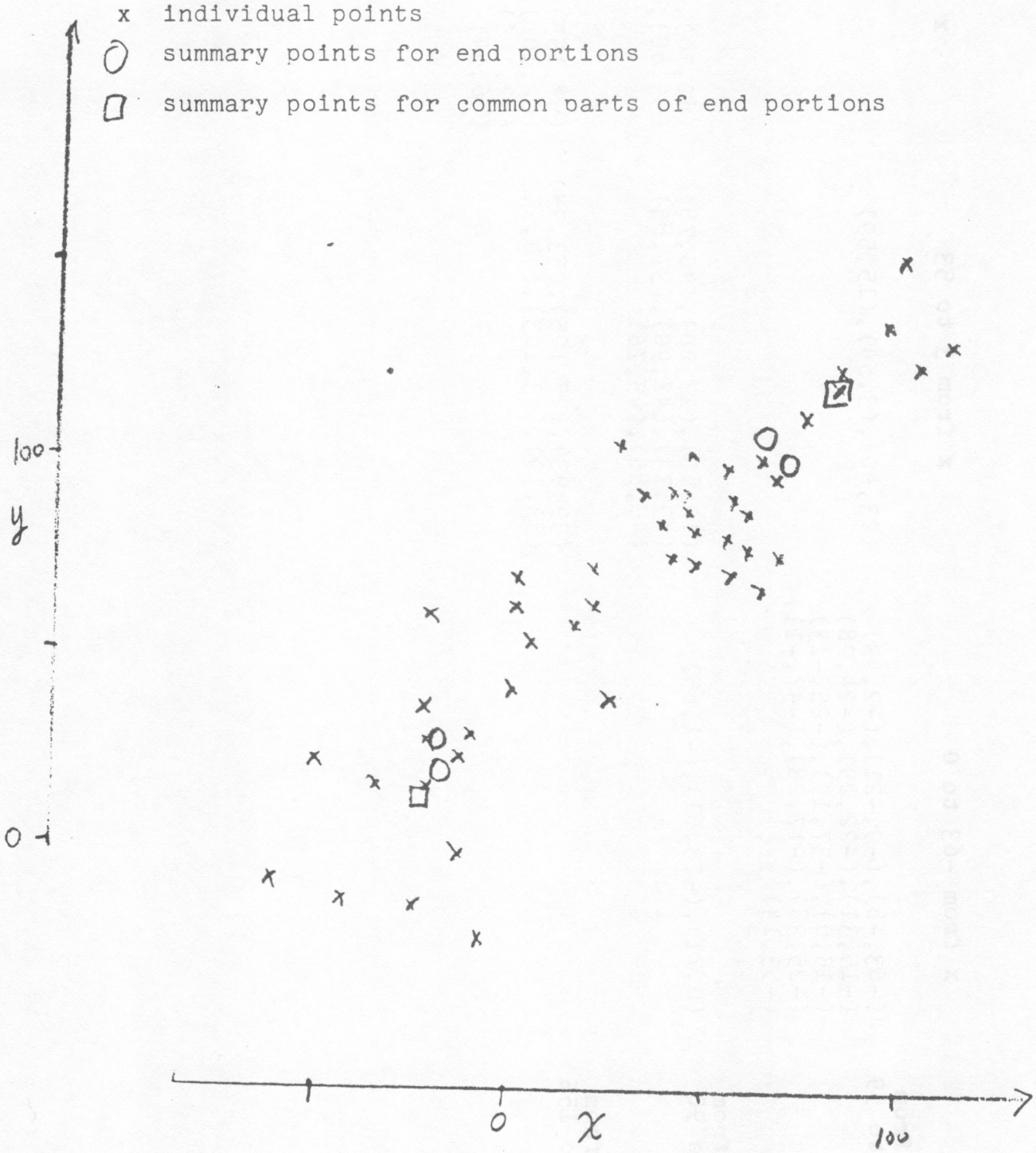


exhibit 11 of chapter 11 (Cont'd)

C) FITTED LINE

$$\text{tilt} = \frac{120.2-14.8}{81 - (-25)} = \frac{105.4}{106} = .994$$

$$y \sim .994x + 39.65$$

D) ROUND NUMBERS LINE

$$y \sim x + 40$$

which translates through:

log motor vehicle deaths $\sim$ log marriages - 1.60

to:

(motor vehicle deaths) $\sim \frac{1}{40}$ (marriages)

Notes:

$x = -400 + 100 \log \text{marriages}$

$y = -200 + 100 \log \text{motor vehicle deaths}$

$-1.60 = \log(1/40)$

exhibit 12 of chapter 11

1960 Populations of 49 States

Used to Fit An Anchored Line

A) POPULATION AND STATE -- in thirds by size

Pop'n (1000's)	State	Pop'n (1000's)	State	Pop'n (1000's)	State
16,782	NY	3,567	Tennessee	1,411	Nebraska
15,717	Calif	3,413	Minnesota	1,302	Arizona
11,319	Penn	3,266	Alabama	969	Maine
10,081	Ill	3,257	Louisiana	951	N. Mexico
9,706	Ohio	3,100	Maryland	890	Utah
9,597	Texas	3,038	Kentucky	859	R. I.
7,823	Mich	2,853	Washington	763	D. C.
6,066	NJ	2,757	Iowa	680	S. Dakota
5,148	Mass	2,535	Conn	674	Montana
4,951	Fla	2,382	S. Car	667	Idaho
4,662	Ind	2,328	Oklahoma	632	N. Dakota
4,556	N. Car	2,178	Kansas	606	N. H.
4,319	Mo	2,178	Mississippi	446	Delaware
3,966	Va	1,860	W. Va	389	Vermont
3,951	Wis	1,786	Arkansas	330	Wyoming
3,943	Ga	1,768	Oregon	285	Nevada
		1,753	Colorado		

B) x and y VALUES from exhibit 12 of chapter 10 -- for same thirds

(110,133)	(45,86)	(3,54)
(98,155)	(37,85)	(0,71)
(81,122)	(39,93)	(-15,31)
(92,128)	(33,93)	(-23,61)
(82,126)	(62,70)	(-17,28)
(95,137)	(42,90)	(-25,-13)
(73,114)	(45,76)	(-9,-21)
(59,88)	(40,78)	(-25,38)
(66,77)	(23,40)	(-22,29)
(25,105)	(59,79)	(-2,43)
(62,102)	(54,83)	(-37,19)
(43,103)	(19,74)	(-15,0)
(53,99)	(56,74)	(-63,8)
(56,94)	(15,59)	(-47,-11)
(40,91)	(19,65)	(-53,14)
(66,98)	(-1,65)	(-24,28)
	(17,60)	

(Cont'd)

It is all too easy to come to believe that measurement is a matter of great depth, or esoteric, or even sacred. Each of these views has misled many people. We would like to avoid as many of them as we can.

Enrico Fermi, the Nobel Laureate physicist, is reliably reported to have once said; "Measurement is just the making of fine distinctions".

Norman Robert Campbell, who thought as hard and well as anyone about the measurements of classical physics wrote: "Now the devising of methods for judging > is the chief problem of the experimental art. For the range of possible methods is enormous: any effect, however remote and indirect, of change in a magnitude provides a possible method; and the most ingenious experimenter is he who can see ways of using very remote effects." (An account of the principles of measurement and calculation: pp. 41-42.)

The one essential of all carefully axiomatized schemes of measurement (see page 1 of Vol. 1 of J. Math. Psychol. for an unCampbellian instance) is the notion of >, of an ability to compare -- and thus to recognize order.

As Campbell puts it on page 8 of the same book: "The conception of a magnitude is inseparable from that of the order characteristic of it. It is natural, therefore, to regard as the same magnitudes, or as magnitudes of the same kind, properties that invariably have the same order."

One basic thing in establishing how a specific property -- for example, tilt -- is to be measured, is to decide

The eye is best at comparing with a horizontal line -- moreover, once the line is untilted, we may be able to increase the magnification with which we look at the result. Even middle-mean arithmetic operates better -- as we have been shown by the change of order of residuals, as compared to the order of y-values -- when we work with residuals.

In practice or in theory we ought to define the tilt of a pattern as the slope of a line which, when subtracted, makes the pattern look untilted.

11E. <u>One-point assays</u> (optional)

Sometimes we want to use a fitted line as a basis for indirect measurement. This is common in chemistry, for example, as problems 2, 3, and 4 of chapter 8 illustrate. The collection of data and the fitting of a "calibration curve" to it is a standard preparation for chemical measurement.

The same is true of the measurement of biological activity: assessing the potency of drugs, of insecticides, and of vaccines provides many standard examples. These are, however, most usually examples of the method of the next section, rather than of the present one, since they routinely use a number of differing doses.

An entirely similar situation can arise in very different circumstances. We have spent much time and space in this chapter on the relation between number of motor vehicle deaths and number of marriages. Let us suppose that the number of motor vehicle deaths was lost for Tennessee, and that we

exhibit 13 of chapter 11

Using Marriages and Fitted Lines to Assess the Number of Motor Vehicle Deaths in Tennessee in 1958

A) USING THE LINE FITTED TO y -- exhibit 12 of chapter 10

Equation:	$y = .811x + 48.5$
x for Tennessee:	45
y from line:	85.3
corresponding number:	713

B) USING THE LINE FITTED TO x -- exhibit 2

Equation:	$x - .978y - 40.8$
Equivalent form:	$y = 1.022x + 41.7$
x for Tennessee:	45
y from line:	87.7
corresponding number:	753

C) USING THE COMPROMISE LINE -- exhibit 11

Equation:	$y = .994x + 39.65$
x for Tennessee:	45
y from line:	84.4
corresponding number:	698

D) USING THE "ROUND NUMBERS" LINE -- exhibit 11

Equation:	$y = x + 40$
x for Tennessee:	45
y for line:	85
corresponding number:	708

E) USING THE ANCHORED LINE -- exhibit 12

Equation:	$y = .915x + 45.23$
x for Tennessee:	45
y for line:	86.4
corresponding number:	731

In such a situation, even-handedness is to be sought by using the anchored line that is anchored by the variable in whose terms we choose to be even-handed.

11F. Multiple-dose assays (optional technique)

Most biologically active materials -- vitamins, drugs, vaccines, etc. -- are measured by biological assay. This means that there is an accepted standard and that the material to be assayed is compared -- usually at more than one dose level -- with the standard -- which also is used at more than one dose -- as to its biological activity -- as shown in animals, plants, or bacteria.

In almost every case, comparison means taking a new lot of animals, plants, or bacteria, dividing them without prejudice among the doses to be tested, measuring their responses and calculating a comparative potency. Variation from one lot to another is so large -- "biological variation" is a common watchword -- that it would not be satisfactory to compare an unknown tested today against a standard tested on some other day.

The first requirement for a technically satisfactory assay is that the unknown "behave like a dilution of the standard". What this means is simple:

- a dose d of the unknown behaves like a dose pd of the standard, where the potency p is the same for all d.

It is better to know how to find some reasonable value for the potency of an unknown than to be unable to do even this. So we shall learn our beginning, thinking it of reasonable value, without forgetting that it is but the first part of a large whole.

* parallel-line assays *

The simplest assays are parallel-line assays. Because the log of a product is the sum of the logs, to say that a dose d of an unknown behaves like a dose pd of a standard is the same thing as to say that a log dose

$$x = \log d$$

of an unknown behaves like a log dose

$$x = \log(pd) = \log p + \log d$$

of a standard.

It is traditional to write

$$M = \log p$$

so that a log dose x of the unknown behaves like a log dose

$$x + M$$

of the standard.

If we have an expression y for the response, so that y is linear in x for the standard

$$y \sim a + bx$$

then for a dose x of the unknown we expect

exhibit 14 of chapter 11

Geometry of An Ideal Parallel-line Assay

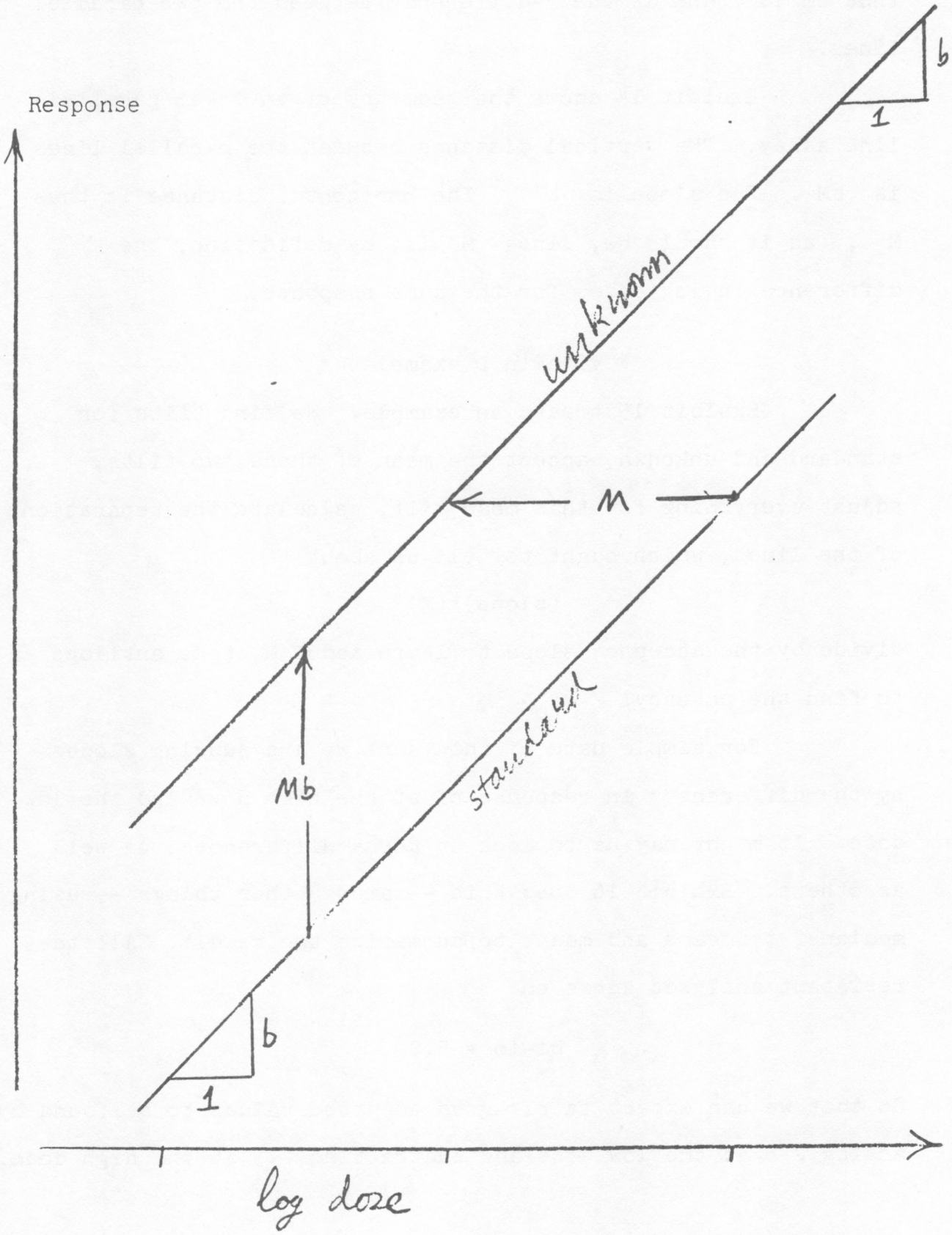


exhibit 16 of chapter 11

Relevant Differences in exhibit 15

	standard			unknown		
Litter	**hi - lo**	**mid**	**mid - ½(hi + lo)**	**hi - lo**	**mid**	**mid - ½(hi + lo)**
1	6	8	3	4	9	4
2	3	4	-3.5	5	5	-0.5
3	8	6	-2	5	6	-0.5
4	1	11	1.5	7	14	4.5
5	7	15	1.5	2	8	-1.0
6	-2	7	1	3	9	1.5
7	9	10	1.5	8	11	2.0
8	-2	4	-6	12	6	-3
9	12	9	1	1	8	2.5
10	9	7	-1.5	6	10	3.0
11	-3	10	-0.5	0	18	3.0
12	7	8	0.5	5	8	-1.5
median	6.0		0.8	5.0		1.8
trimean	5.2		0.3	4.7		1.4
(mean)	(4.6)		(0.5)	(4.8)		(1.2)

exhibit **17** of chapter 11

Adjusted Responses, Medians, Comparisons

A) MAIN CALCULATION -- 2.5 added at low dose, 2.5 subtracted at high dose

	Adjusted responses for standard				Adjusted responses for unknown				
Litter	**2.5**	**5.0**	**10.0**	**Median**	**2.5**	**5.0**	**10.0**	**Median**	**Comparison***
1	4.5	8	5.5	5.5	5.5	9	4.5	5.5	0
2	8.5	4	6.5	6.5	5.5	5	5.5	5.5	-1
3	6.5	6	9.5	6.5	6.5	6	6.5	6.5	0
4	11.5	11	7.5	11.	8.5	14	10.5	10.5	-0.5
5	12.5	15	14.5	14.5	10.5	8	7.5	8	-6.5
6	9.5	7	2.5	7.	8.5	9	6.5	8.5	1.5
7	6.5	10	10.5	10	7.5	11	10.5	10.5	0.5
8	13.5	4	6.5	6.5	5.5	6	12.5	6	-0.5
9	4.5	9	11.5	9	7.5	8	3.5	7.5	-1.5
10	6.5	7	10.5	7	6.5	10	7.5	7.5	0.5
11	14.5	10	6.5	10	17.5	18	12.5	17.5	7.5
12	6.5	8	8.5	8	9.5	8	9.5	9.5	1.5

Comparison = difference of medians (unknown - standard).

B) CENTRAL VALUES of COMBINATION

ordered combinations

-6.5	
-1.5	
-1	-0.75H
-0.5	
-0.5	
0	0.0M
0	
0.5	
0.5	1.0H
1.	
1.5	
7.5	

Mean = 0.12
Trimean = 0.0
Median = 0.00

$$\text{mid} - \frac{1}{2}(\text{hi}+\text{lo})$$

in search of systematic effects.

The first approach does this separately for each litter and each material (standard or unknown), and then summarizes the results asking whether these entries seem to lean either to the + side (corresponding to curvature bulging upwards) or to the - side (corresponding to curvature bulging downwards). Exhibit 16 summarizes the results. Medians, trimeans and means all show a tendency to lean to the + side.

The second approach looks for curvature as likely to be S-shaped, the curvature expected for too high doses being opposite to that expected for too low doses, since both come from a flattening off. (Reader should make a sketch.) The natural way to do this is to plot the same quantity against some measure of overall response and look for a trend. (The first approach asked whether the overall level of this same plot seemed to be zero.)

It is easy,and reasonable, to take the mean response at all three doses as a measure of overall response. Exhibit 18 does this. There may be an indication of an upward trend, but again not pronounced enough to give us pause. The lowest point -- for the standard applied to a rat from litter 8 -- appears discrepant, but we have no basis for further inquiry.

The third approach looks for curvature as a moving tilt rather than as a local bend. It asks if the apparent tilts seem to drift as the overall response alters. The simple measure of tilt is the difference of response

hi-lo

for the extreme doses. Exhibit **19** plots these differences against the response to the mid-dose. Again we see nothing distinctive. In this data, any deviations from linearity are well concealed.

* vitamin C example *

A second example illustrates the need for care and plotting, even in neatly balanced multiple-dose assays. Exhibit **20** gives the basic numbers. Exhibit **21** plots the midmean points. As Bliss pointed out, the high dose of orange juice clearly went beyond the linear range, and should not be considered.

The slope, expressed as increase in odontoblast reading for a doubling of the dose, is, using the other midmean values:

$\frac{25.6-7.7}{2} = \frac{17.9}{2} = 9.0$ for ascorbic acid

10.9 for orange juice

9.4 combined

exhibit 20 of chapter 11

Assay by Length of Odontoblasts in Guinea Pigs
Vitamin C Activity of Orange Juice Vs. Ascorbic Acid

A) DATA* and CENTRAL VALUES -- 10 guinea pigs/dose

	Ascorbic Acid			Orange Juice		
Dose (mg/day**)	**0.5**	**1.0**	**2.0**	**0.5**	**1.0**	**2.0**
	4.2	13.6	18.5	8.2	14.5	22.4
	5.2	14.5	21.5	9.4	19.7	23.0
	5.8	15.2	23.3	9.7	20.0	24.5
	6.4	15.5	23.6	9.7	21.2	24.8
	7.0	16.1	25.5	10.0	23.3	25.5
	7.3	16.1	26.4	14.5	23.6	26.4
	10.0	17.3	26.7	15.2	25.2	26.4
	11.2	17.3	29.1	16.1	25.8	27.3
	11.2	18.8	32.1	17.6	26.4	29.4
	11.5	22.1	33.9	21.5	27.3	30.9
midmean	7.7	16.2	25.6	12.4	23.3	25.8
trimean	7.7	16.2	26.1	12.6	23.2	25.9
mean	8.0	16.6	26.1	13.2	22.7	26.1

* Entries are: Odontoblast readings less 20.

** Doses are in terms of ascorbic acid content by chemical analysis.

B) SOURCE

E. W. Crampton 1947. J. Nutrition 33:491. Used by C. I. Bliss at page 500 of Statistical Methods in Vitamin Research, which is ...

Note that the double length difference in x for ascorbic acid warrants four ($= 2^2$) times the weight.

Adjusting midmeans to middle doses, we find:

Ascorbic acid	**Orange juice**
7.7 + 9.4 = 17.1	12.4 + 9.4 = 21.8
16.2	23.3
25.6 - 9.4 = 16.8	
mean = 16.7	mean = 22.6

$$M = \frac{22.6-16.7}{9.4}(0.3) = .19$$

$$\text{potency} = \text{antilog } .19 = 1.5^{+}$$

Thus orange juice seems to be rather more than 3/2 as effective as the amount of pure ascorbic acid found in the orange juice by chemical analysis.

The simplest forms of calibration involve fitting straight lines with y = effect, x = what is to be measured. These lines are then used to turn y's into x's. Since we must be prepared to face criticism as to our even-handedness in measuring things that are all small -- or all large -- we routinely use lines fitted to y for our calibrations.

The measurement of biological activity illustrates the situation where our measuring device is not very stable, where -- as a result -- we need to include a calibration with every measurement. Here it is common to use both standard and unknown at two or more doses, thus allowing the fitting of two lines, once we have found a suitable way of expressing the response.

exhibit 22 of chapter 11

An Extreme Situation

A) LOW-x PORTION ≡ LOW-y PORTION

	x	y	y-x	-.08x	diff	x-y	.08y	diff
	-63	-8	55	5.0	50.0	-55	-.6	-54.4
	-9	-21	-12	.7	-12.7	-12	-1.7	13.7
	-2	43	45	.2	44.8	-45	3.4	-48.4
	-15	31	46	1.2	44.8	-46	2.5	-48.5
	-22	29	51	1.8	49.2	-51	2.3	-53.3
	-24	28	52	1.9	50.1	-52	2.2	-54.2
	-15	0	15	1.2	13.8	-15	0.0	-15.0
Mean	-21.4	14.6	(36.0)					
Trimean	-16	20	40		(38.4)	-40		(-46.5)
Midmean	-17	19	47		46.3	-47		-50.1

B) MID-x PORTION ≡ MID-y PORTION

	x	y	y-x	-.08x	diff	x-y	.08y	diff
	19	65	44	-1.5	45.5	-44	5.2	-49.2
	17	60	43	-1.4	44.4	-43	4.8	-47.8
	40	78	38	-3.2	41.2	-38	6.2	-44.2
	19	74	55	-1.5	56.5	-55	5.9	-60.9
	42	90	48	-3.4	51.4	-48	7.2	-55.2
	37	85	48	-3.0	51.0	-48	6.8	-54.8
	45	86	41	-3.6	44.6	-41	6.9	-47.9
Mean	31.3	76.9						
Trimean	34	77	44		(47.2)	-44		(-50.4)
Midmean	32	79	45		47.0	-45		-50.6

C) HIGH-x PORTION ≡ HIGH-y PORTION

	x	y	y-x	-.08x	diff	x-y	.08y	diff
	98	155	57	-7.8	64.8	-57	12.4	-69.4
	66	98	32	-5.3	37.3	-32	7.8	-39.8
	92	128	36	-7.4	43.4	-36	10.2	-46.2
	62	102	40	-5.0	45.0	-40	8.2	-48.2
	73	114	41	-5.6	46.6	-41	9.1	-50.1
	110	133	23	-8.8	31.8	-23	10.6	-33.6
	82	126	44	-6.6	50.6	-44	10.1	-54.1
	83.3	122.3						
	82	122	39		(45.0)	-39		(-47.6)
Midmean	82	123	39		45.0	-39		-48.2

(Cont'd)

exhibit 22 of chapter 11 (Cont'd)

H) MIDMEAN TILT OF x ON y

	at y = 19	at y = 79	at y = 123	hi - lo	same / 104	(trial)
x	-17	32	82	99	.95	(1.0)
x-y	-47	-45	-39	8	.08	(.08)
x-1.08y	-50.1	-50.6	-48.2	1.9	.02	(take it)
(.02y)	(0.4)	(1.6)	(2.5)			
(x-1.10y)	(-50.5)	(-52.2)	(-50.7)	$\frac{\text{sum}}{3} = -51.1$		

$$x \sim 1.10y - 51.1$$

J) COMPARISON OF LINES

Fitted too	Using	y as function of x	x as function of y
y	tri	**.99x + 41.3**	1.01y - 41.7
x	tri	.99x + 41.3	**1.01y - 41.7**
y	mid	**.91x + 46.4**	1.10y - 51.1
x	mid	.91x + 46.4	**1.10y - 51.1**
y	mean	**1.03x + 39.3**	.97y - 38.2
x	mean	1.03x + 39.2	**.97y - 38.1**

Note: Differences in coefficients for last two lines are due only to rounding.

*****

11P. Problems

1. Bliss (p. 503 of Statistical Methods in Vitamin Research) gives data by Burkholder on a microbiological assay by riboflavin by the method of Light and Clarke. Exhibit 23 gives the necessary numbers. What is an apparent potency? What oddities, if any, can you find? Discuss.

2. J. O. Irwin (1944, Journal of Hygiene 43, 291, see also Bliss, page 512) reported the results of a collaborative assay of Vitamin A β-naphoate by the weight gain of male rats. Exhibit 24 gives the necessary numbers. What is an apparent potency? Any oddities? Discuss your results.

3. Bliss (page 518 of Statistical Methods for Vitamin Research) cites data of the E. I. duPont de Nemours and Co., Inc. on the assay of two vitamin D oils against U.S.P. reference Cod Liver Oil No. 2 by the line test in rats. Exhibit 25 gives the necessary numbers. What is an apparent potency? Any oddities? Any discussion?

4. K. H. Coward (1947, Biological Standardization of the Vitamins, Second edition. Balliere, Tindall and Cox. See also C. I. Bliss 1940, J. Amer. Statist. Assoc. 35:498, and Bliss, page 527 of Statistical Methods in Vitamin Research) gave figures on the ash content of dry bone observed in an assay of vitamin D in rats. Exhibit 26 repeats some of the numbers. What is an apparent potency? Any oddities? Any discussion?

exhibit 24 of chapter 11

Gain in Weight of Male Rats

	Standard		Unknown		(Relative doses)
Litter	**1**	**2**	**1**	**2**	
1	38	44	31	42	
2	57	62	50	64	
3	29	45	31	52	
4	23	38	16	34	
5	26	59	29	44	
6	32	40	24	47	
7	43	51	30	40	
8	39	62	29	42	
9	51	74	35	49	
10	64	66	43	52	

exhibit 26 of chapter 11

Ash Content of Dry Bone

(Vitamin D assay)

A) LOG WEIGHT OF ASH

	Standard			Unknown		
Litter	**0.025**	**0.05**	**0.1**	**0.2**	**0.4**	**0.8**
1	.207	.494	.633	.396	.537	.702
2	.316	.459	.640	.467	.590	.760
3	.377	.606	.751	.511	.678	.880
4	.281	.450	.720	.449	.619	.820
5	.318	.431	.674	.423	.464	.778
6	.262	.453	.708	.498	.614	.778

Doses of standard: Units of international standard Vitamin D.

Doses of unknown: mg/day of unknown cod liver oil.